THE ACTION OF ELECTRICAL STIMULATION AND OF CERTAIN DRUGS ON CARDIAC NERVES OF THE CRAB, CANCER IRRORATUS

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Introduction

The nervous regulation of the decapod crustacean heart has been studied for over a century, yet the nature of the mechanisms involved are still obscure. The nerves concerned are minute, and only in recent years has a picture been obtained of the anatomical relationships of the cardio-regulatory nerves to the heart and its ganglion cells.

Inhibitory cardiac nerves in crayfish were early indicated or demonstrated by several investigators including Dogiel (1876, 1877), Yung (1878), and Plateau (1878, 1880), whose work showed in a general way that these nerves arise from the anterior part of the thoracic ganglionic chain or mass. Dogiel believed that nerves arising anterior to the sternal artery and running to the pericardium and abdominal extensor muscles were inhibitory, but as his only points of electrical stimulation were the pericardium and the thoracic nerve cord between the bases of the second and third leg nerves it does not appear that he traced the inhibitory nerves in detail. In view of frequent misquotations it is well to note that "Dogiel's nerve" was never claimed to run along the sternal artery or actually to enter the heart. Inhibitory cardiac nerves in the crayfish have recently been traced by Wiersma and Novitski (1942).

Definitive information on the course of inhibitory cardiac nerves in crabs rests upon the work of Jolyet and Viallanes (1892, 1893), Conant and Clark (1896), and Bottazzi (1901). Conant and Clark demonstrated most clearly in *Callinectes* that the inhibitory nerves arise as a single pair close to the bases of the recurrent cutaneous nerves, and run with these large nerves anterodorsally from the thoracic ganglion, eventually separating and joining the cardio-accelerator nerves on each side to form the lateral pericardial plexus. The entrance of the inhibitory nerves into the heart was not observed by Conant and Clark, but the observations of Alexandrowicz (1932), Heath (1941), and the writer are in agreement that the cardio-regulatory nerves enter the crab heart as a single dorsolateral pair of delicate strands, containing very few fibers.

Interest in cardio-accelerator nerves at first centered about "Lemoine's nerve," which was described in the crayfish by Lemoine (1868) as arising from the stomatogastric nervous system on the anterior dorsal wall of the stomach. This nerve, which is extremely fine, was reported to run beneath the ophthalmic artery and to break up as it entered the heart. In view of later misquotations, it is well to note

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that Lemoine did not derive his cardiac nerve from the cerebral ganglia, nor did he produce any effects on the heart by stimulation of the "brain." The positive results of stimulation of "Lemoine's nerve" reported by several authors (Lemoine, 1868; Yung, 1878; Plateau, 1880) were possibly a reflex phenomenon. More recent workers, including Jolyet and Viallanes (1892, 1893), Conant and Clark (1896), Wiersma and Novitski (1942), have not found "Lemoine's nerve" to be a cardio-accelerator. Vital staining studies by Alexandrowicz (1932), Heath (1941) and the writer show that this nerve in the crab innervates the anterior valves of the heart, and does not pass to heart ganglion or muscle. Jolyet and Viallanes (1892, 1893), confirmed by Conant and Clark (1896), showed that the cardio-acceleratory nerves in crabs consist of two pairs arising dorsal to the bases of the nerves to the third maxilliped and the first leg. The two acceleratory nerves on each side join the single inhibitory nerve to form the lateral pericardial plexus, from which the acceleratory nerves pass into the heart in the same thin strand which carries the inhibitors.

The cardiac ganglion (intrinsic ganglion) of the crab heart, as in other marine decapods, contains about nine cells and sends its fibers in a complex but regular pattern throughout the heart (Alexandrowicz, 1932). It is innervated by the single pair of nerves carrying inhibitory and excitatory fibers. The exact relationship of each type of fiber to the heart ganglion and heart muscle is not fully understood. Alexandrowicz and others have observed that the fibers entering the heart are of two sizes, the larger of which make synaptic contact with processes of the ganglion cells, while the smaller fibers pass to the muscle. Alexandrowicz suggests that the thicker fibers are inhibitory, the thinner excitatory. However, in their studies of the multiple innervation of crustacean limb muscles, van Harreveld and Wiersma (1939) have found that the inhibitory fiber is thinner than the motor fibers to the same muscle, while Wiersma and Novitski (1942) have shown that the cardio-acceleratory nerves in the crayfish probably act upon the heart ganglion rather than on muscle.

Pharmacological studies have indicated that a cholinergic nervous mechanism is concerned in the initiation of heartbeat in higher crustaceans (Welsh, 1939a, 1939b; Davenport, Loomis, and Opler, 1940; Davenport, 1941, 1942; Prosser, 1942 and others). The isolated decapod heart is stimulated by acetylcholine, an effect augmented by eserine and blocked by atropine. While it is generally agreed that the intrinsic ganglion has a pacemaker function, and that it is stimulated by acetylcholine (Welsh, 1942), attempts to determine whether acetylcholine acts in a muscarine-like or in a nicotine-like fashion on the ganglion or muscle of the isolated heart (Davenport, 1941, 1942) have revealed little concerning the manner of action of the regulatory nerves which reach the heart from the central nervous system.

In pharmacological studies of the isolated heart it has generally not been possible to tell at what sites the drugs used are actually taking effect. For instance, the rate of the isolated crustacean heart is quickened by acetylcholine. This effect is probably due to the action of the drug on the heart ganglion, but the possibility of a direct action on the muscle remains. The sensitivity of the ganglion to acetylcholine does not prove that the neurons of the ganglion are themselves cholinergic. They might be cholinergic, adrenergic, or of some still unknown nature. It is helpful to recall that in vertebrates cholinergic preganglionic fibers may stimulate either

cholinergic or adrenergic postganglionic neurons in the autonomic nervous system. Thus the situation in the isolated crustacean heart is more complex than it may appear at first glance. When a drug is applied to the whole heart, it undoubtedly has some effect on muscle as well as on the ganglion, and if we admit the possibility of its stimulating the remnants of regulatory nerve still within the heart, we see further complications, not only because we may not be able to identify the effects of the regulatory nerves in the total response, but also because the simultaneous stimulation of these opposing nerves could produce conflicting effects. Since the completion of the work reported here, Wiersma and Novitski (1942) have shown that perfused acetylcholine and excitatory nerve stimulation produce similar effects in the crayfish heart, and that eserine augments the effect of nerve stimulation upon heart rate. These observations may be considered as excellent evidence for the cholingeric nature of the cardio-accelerator nerves and for the termination of these nerves upon the ganglion cells rather than on muscle. However, it is still not known at what point in the heart the inhibitory nerves act. Previous studies by the writer on nervous inhibition of the heart of *Panulirus* (Smith, 1940–41) led to the conclusion that the cardiac ganglion was possibly cholinergic, but that the inhibitory mechanism was not of the "muscarine-like" cholinergic type.[2]

In view of the scarcity of direct evidence on the nature of the regulatory heart nerves it was felt that further study of the effects of certain drugs on the action of these nerves in a semi-intact preparation might yield information regarding their nature and mode of action. It was originally hoped that this work could include an electrical study of the heart ganglion when the heart was being inhibited or excited by nervous action, in order to clarify the role of the heart ganglion in nervous control, but, unfortunately, exigencies of 1942 did not permit electrical studies to be made.

Throughout this work I received constant encouragement and much helpful criticism from Dr. John H. Welsh, to whom I wish to express my gratitude.

Material and Methods

The eastern rock crab, *Cancer irroratus,* was found to be of suitable size and hardiness for winter laboratory use if maintained under cold conditions. By suitable cannulation, the heart was perfused *in situ* with physiological solutions or drugs. Heart action was recorded kymographically, while stimulation was applied to the inhibitory and to the excitatory nerves by means of two sets of mechanically manipulated electrodes which were left in place upon the nerves through the course of an experiment. Exposure of the thoracic nerve mass from beneath gave access to these nerves close to their point of origin, at a considerable distance from the heart. Stimulation was supplied either by a pair of Harvard inductoria, or by a thyratron stimulator (modified after Delaunois, 1939) giving repetitive shocks at controllable frequencies and voltages.

Perfusion fluid was made up following Cole's (1940) analysis of the blood of *Cancer borealis.* (An analysis of the blood of *C. irroratus,* generously carried out by Dr. Cole, showed that the composition of the blood of this species is similar to to that of *C. borealis.*)

[2] The statement in the original report (Smith, 1940–41) that, "the inhibitory mechanism is not of the cholinergic type," was not justified by experimental results, and should be in the less sweeping form, "not of the muscarine-like cholinergic type."

NaCl	0.506 M	1000 parts			
KCl	0.506 M	26.1 parts	$MgCl_2$	0.506 M	8.7 parts
$CaCl_2$	0.506 M	26.1 parts	$MgSO_4$	0.506 M	39.1 parts

To each liter of solution was added 17.6 cc. of 0.5 M boric acid and 0.96 cc. of 0.5 M NaOH as a buffer.

The crab heart perfused *in situ* exhibits a steady beat which may be slow or rapid, depending upon the temperature and the conditions of the dissection and perfusion. For best results, all the supporting ligaments of the heart should be intact, although a fairly good beat may often be obtained when the posterodorsal ligament has been severed. A low temperature (10° – 15° C.) is much more favorable than room temperature at any season. The pressure within the heart, which depends upon the rate of perfusion as well as upon the integrity of the heart and its valves, largely controls the amplitude and rate of beat. In diastole the heart is stretched horizontally by its elastic supporting ligaments until it presents a broad, flat or slightly depressed dorsal surface. In systole, the internal pressure causes the dorsal surface to bulge upwards as the heart passes from a flat to a more rounded cross-section, while at the same time, the rear wall of the heart bulges posteriorly. This latter motion was recorded in the tracings as a sharp upward deflection of the writing lever.

With insufficient internal pressure, the heartbeat is weak and irregular, or may even cease. Too great pressure causes a marked increase in amplitude and rate of beat. In this work, the perfusion rate was adjusted until the heart was beating steadily, and not showing more than slight passive swelling between beats. Sudden increases in the rate of perfusion produce an increase in the amplitude and rate of beat strikingly similar to that caused by the excitatory nerves. That this is not a reflex phenomenon involving these nerves can be shown by its continuance after complete removal of the central nervous system. On the other hand, a decrease in the pressure exerted within the heart by the perfusion fluid may cause slowing or cessation of beat. These results of internal pressure changes will be found helpful in explaining certain after-effects of nervous excitation and inhibition.

Effects of Faradic Stimulation of Cardio-Regulatory Nerves

Nervous inhibition

The inhibitory nerve to the heart was stimulated close to the basal portion of the recurrent cutaneous nerve, just forward of the ventral thoracic ganglion (see Fig. 1). With the ventral approach to the inhibitory nerves, there is little danger of confusion from accidental stimulation of the excitatory nerves. In this work, stimuli only slightly above threshold have been used, and in properly set up preparations no effects of any spread of current from the point of stimulation have been noted.

With inductorium stimulation, cardiac inhibition is usually complete, there being only a very narrow range in which partial inhibition can be obtained. For this reason, the inductorium is useful mainly to determine the intensity threshold for complete inhibition. The inhibition obtained by these means is an abrupt cessation of heartbeat when the intensity threshold is reached. This persists for ten to sixty seconds if the stimulus is continued, after which "escape" beats occur, leading to restoration of a normal beat. If the stimulus is discontinued while the heart is stopped, the beat is restored immediately, without perceptible inhibitory after-

effect. In a few cases, the first beats after inhibition are of increased amplitude or rate. Observation has shown that during the period of inhibition, the continued inflow of fluid sometimes stretches the heart passively, as indicated by a rise in the traced record. When a stretching of the muscle occurs, the effect at the restoration of beat is the same as that resulting from an increase in perfusion rate, hence the heart responds by an augmentation of beat. In cases where the rate of perfusion is low, or when the heart stopped in diastole still tends to drain naturally, there is no evidence of distention, and as a result, no stimulatory after-effect of inhibition.

Nervous excitation

The two pairs [3] of excitatory nerves arise dorsal to the bases of the nerves to the third maxillipeds and the chelipeds respectively (see Fig. 1). Stimulation of these nerves in the vicinity of the heart is not effective because of interference from the nearby inhibitory fibers, but they are easily stimulated as they leave the thoracic ganglion. Commonly, the electrodes were placed to the rear of either of the large nerves mentioned above, dorsal to whose roots the heart nerves arise. However, because of the fact that the inhibitory nerves arise only slightly anterior to the excitatory, it is advisable to sever the inhibitory nerve on the side where the excitatory nerves are being stimulated. Excitation caused by faradic stimulation does not show a sharp threshold. It develops gradually with increasing intensity of stimulation, and soon reaches a maximum. One cannot well speak of "partial excitation." It would have been desirable to establish a quantitative measure of the amount of excitation produced, but because of the great variation in the character of the response in different hearts no reliable method was devised. According to the condition of the valves after the manipulation attending cannulation, the accelerated heart may literally pump itself dry, or it may retain fluid and increase the internal pressure. With these and possibly other variables operating to modify the picture of nervous augmentation of heartbeat, faradic stimulation of the excitatory nerves may produce an increase either in rate or amplitude, or in both. The after-effect of excitation is likewise variable, but in contrast to inhibition, excitation commonly shows a stimulatory after-effect, which in some cases lasts for several minutes after stimulation has ceased. This is most noticeable when a preparation is fresh.

A factor which may act to conceal a stimulatory after-effect, and which may even result in a depression, is the tendency of some hearts to pump themselves dry when accelerated, leading to temporary cessation of beat following the period of excitatory stimulation. Nevertheless, the number of times that excitation of the heart has resulted in an unquestionable after-effect in these experiments, as well as in those of earlier workers (Bottazzi, 1901; Conant and Clark, 1896), makes it appear that this phenomenon is a characteristic feature of nervous excitation of the crab heart, as a result of faradic stimulation.

That there is an upper limit to the effectiveness of the excitatory nerves is shown by their inability to produce a state of tetanic contraction in the heart, although the heart may readily be tetanized by direct stimulation, while acetylcholine

[3] No excitation of the heart by stimulation of "Lemoine's nerve" has been noted in this work

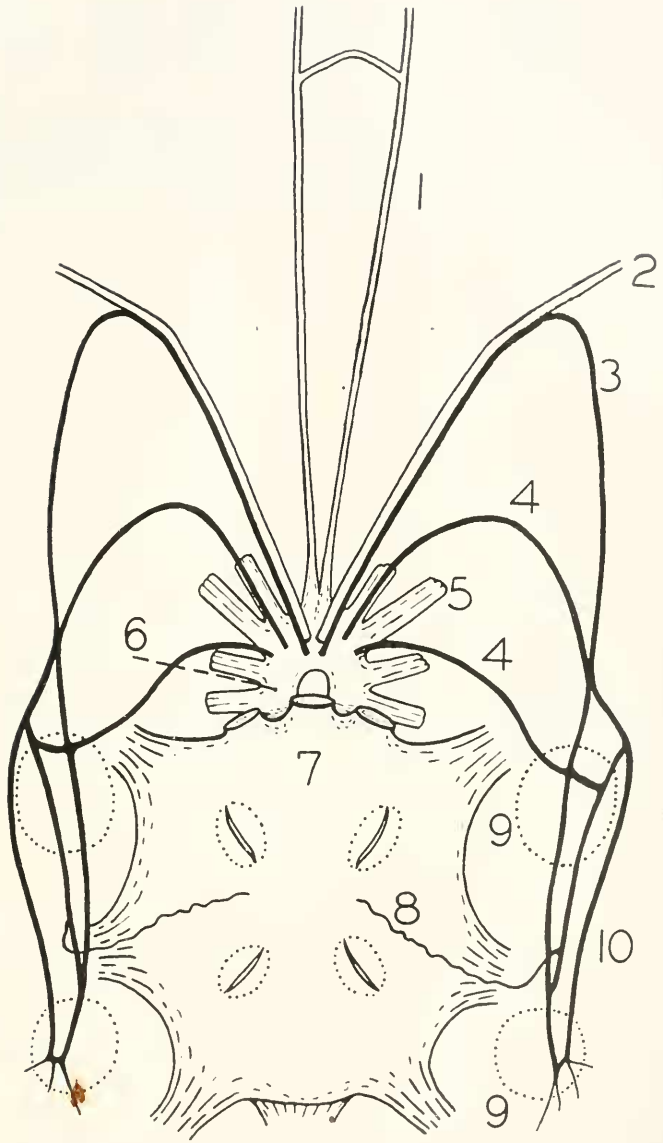

FIGURE 1. Schematic representation of the innervation of the heart of *Cancer* in dorsal aspect, with the thoracic ganglion shown as if moved forward slightly. Approximately to scale, but with cardiac nerves and pericardial plexus drawn with disproportionately heavy lines. (*1*) Circumoesophageal connective, (*2*) recurrent cutaneous nerve, (*3*) inhibitory cardiac nerve, (*4*) (*4*) excitatory cardiac nerves, (*5*) nerve to cheliped, (*6*) thoracic nerve mass, (*7*) heart, (*8*) dorsal nerve entering heart, (*9*) (*9*) branchio-cardiac orifices, (*10*) lateral pericardial plexus.

in high concentrations has been shown to cause an accelerated beat leading to a tetanus (Welsh, 1939a, b; Davenport, 1941).

Such results indicate that the extrinsic excitatory nerves do not directly control the rate of heartbeat, but must act to alter the rate of spontaneous activity in the pacemaker mechanism of the heart. Presumably the effectiveness of these nerves lies in their ability to alter the conditions determining the activity of the cardiac ganglion without being able to force the latter beyond certain limits.

It is commonly stated that the crustacean heart muscle, unlike the vertebrate heart, shows no absolute refractory period. This generalization holds when the heart is stimulated via the excitatory nerves, since cardiac excitation may commence at any phase of the beat, resulting in a well-marked summation. In this respect, the crustacean heart reacts rather more like skeletal muscle than like vertebrate cardiac muscle.

Effects of Frequency of Stimulation Upon the Cardio-Regulatory Nerves

In an effort to obtain a clearer picture of the action of the cardio-regulatory nerves, they were stimulated over a wide range of frequencies, employing a thyratron stimulator which delivered repetitive shocks over a range of 10 to 1,800 per second, with voltage adjustable from 0 to 10 volts. This stimulator could be connected at will to either of the two sets of electrodes in use, allowing comparable studies to be made on excitatory and inhibitory nerves in the same preparation over the same period. Both electrodes were fixed in place and undisturbed through an experiment.

Preliminary experiments showed that at very low or high frequencies the voltage required to produce a response was higher than at intermediate frequencies. Accordingly, a series of trials was made to determine the threshold voltage at various frequencies. Frequency-intensity threshold curves were plotted, showing the changes in intensity threshold over a wide range of stimulation frequencies, and the frequency limits above and below which stimulation of the heart nerves produced no effect. The results of a typical experiment are shown in Figure 2.

The greatest difficulty in this method is the problem of recognizing the onset of response in the heart at low or high frequencies outside of the optimum range. Over most of the frequency range, inhibition is total and begins abruptly, so that the threshold is easily determined, but at low frequencies, inhibition may first appear as a slight, gradually increased slowing of the heart, which may or may not cease beating abruptly when a higher voltage is reached. In such cases, two thresholds must be noted, one for partial, and one for total inhibition. In the case of excitation, while the onset of response is sharp over most of the frequency range, there may be a gradual acceleration at low frequencies that is especially hard to detect. It has not been possible to establish any criterion of "partial" excitation, hence the first noticeable increase in rate or amplitude has been taken as the threshold. Obviously, in all tests of this sort it is important to raise the intensity at a uniform rate, ceasing to raise the intensity as soon as results are observed on the kymograph tracing. Further to lessen the subjectivity of observations, tests were carried out in a planned series without stopping to verify individual readings. The series of frequencies were then passed through in the reverse direction in such a way that descending frequency settings alternated with those of the ascending series. No curves were con-

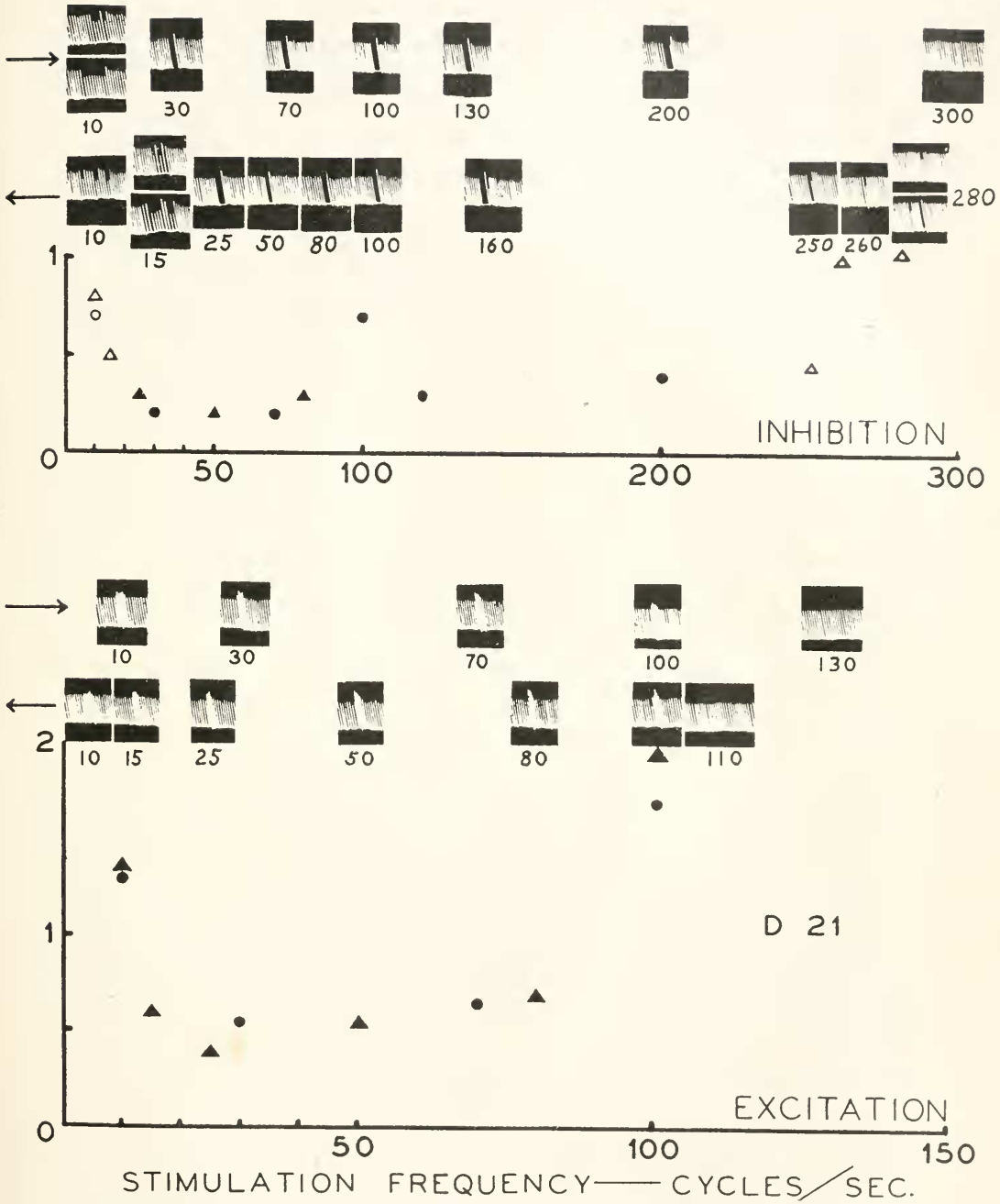

FIGURE 2. Frequency-intensity threshold curves for stimulation of the cardiac inhibitory and excitatory nerves, with tracing of the heart response at each frequency. Circles on the curve represent determinations made on an ascending scale of frequencies; triangles those on a following descending frequency series. Both curves obtained from the same animal (D–21), with tests made alternately on inhibitory and excitatory nerves. Open symbols indicate thresholds for partial inhibition at frequencies where no total inhibition could be obtained.

sidered valid if the electrodes had to be moved during the course of the experiment. Certain generalizations may be made regarding the effects of the frequency of stimulation on the cardio-regulatory nerves.

The excitatory nerves

1. The excitatory nerves show a low intensity (voltage) threshold for stimulation over a wide range of frequencies.

2. The lower frequency limit for effective stimulation of the excitatory nerves is lower than that for the inhibitory nerves. At frequencies as low as 10 per second there is often only a very slight rise in the threshold intensity of stimulation.

3. The upper frequency limit of effectiveness of stimulation of the excitatory nerves is variable, usually between 100 and 300 per second, but in some cases ranging well above 300 per second. In such cases it is unlikely that the nerve is actually discharging at such a high rate. The usual upper frequency limits are in agreement with the findings of Bogue and Rosenberg (1936), who report that in the leg nerves of *Maia* a large proportion of the motor fibers respond regularly to stimuli delivered at the rate of 200 per second, but that at 400 per second the fibers cease to respond in phase.

4. In the lower range of stimulation frequencies, higher voltages are required to stimulate the excitatory nerves, and less effect is produced upon the heart than when higher stimulation frequencies are employed. Raising the intensity of low-frequency stimulation does not produce the marked increase in response of the heart that can be produced by raising the frequency. This finding is consistent with the small number of nerve fibers innervating the heart and with the marked facilitation exhibited by crustacean nerves.

The inhibitory nerves

1. There is a frequency range from about 15–20 stimulating shocks per second up to 100 or 200 per second in which inhibition is complete at or near the threshold voltage of inhibitory nerve stimulation.

2. At a lower range of frequency, inhibition becomes only partially effective. In some preparations, stimulation of the inhibitory nerve at 10 per second has no visible effect on the heart, regardless of the stimulus intensity.

3. At the upper range of effective frequencies, stimulation of the inhibitory nerve often causes only partial inhibition. There is so much variation in the upper limit of effectiveness that no significance can be attached to its value in the individual preparation.

The effect of aging of the preparation upon the frequency-intensity threshold curves for inhibitory and excitatory nerves

Since in the experiments with perfused drugs, to be described below, it was necessary to run repeated series over periods of several hours, a number of undrugged preparations were tested repeatedly in order to determine the effects of fatigue. There is exhibited in successive runs a slight raising of the intensity threshold, associated with a narrowing of the frequency range over which stimulation of the heart nerves is effective. This is most marked at the upper end of the

frequency range, where no certain significance can be attached to it. The lower end of the frequency curve shows much less tendency to shift with aging than does the upper. For this reason, in the experiments with drugs, the main attention was directed to that portion of the frequency curve lying below 100 per second.

Simultaneous Stimulation of Inhibitory and Excitatory Nerves

Using two inductoria, excitatory nerves have been stimulated during the course of nervous inhibition. In all cases where equal intensities have been used, inhibition remains dominant, and is not interrupted even when much stronger intensities of stimulation are applied to the excitatory nerve. Also, if an inhibitory nerve is stimulated during a period of nervous excitation, inhibition readily interrupts the excitation. But since a stronger inhibitory stimulus is required to stop a heart already under the influence of the excitatory nerve than is required to stop the unstimulated heart, it would seem that to a certain extent the inhibitory and excitatory nerves are each able to modify the effects produced by the other.

Further work, especially electrical studies of the ganglionic pacemaker within the heart, is needed before it can be said whether or not the "blending" of the actions of inhibitory and excitatory heart nerves means that these opposing nerves affect the same portion of the pacemaker system.

Effects of Strychnine upon Nervous Excitation and Inhibition of the Crab Heart

A number of tests have been made of the action of strychnine upon the cardiac inhibitory and excitatory nerves. Because strychnine in the concentrations necessary to affect the regulatory nerves has been found to affect the heartbeat adversely, about half of the experiments have been inconclusive. This is especially true in regard to studies on inhibition, since a heart that has stopped beating cannot be further inhibited, even though it can frequently be made to beat by stimulation of the excitatory nerves. The effects of strychnine on the character of the heartbeat generally follow a sequence that is passed through more or less quickly depending upon the concentration of drug applied. There is first a moderate increase in rate and amplitude of beat, giving way to a phase of grouped beats, in which groups of 2–8 beats are separated by short (2–5 second) pauses. With a decrease in the number of beats in a group, a state is reached in which the heart records single spike-like beats at intervals of 10–30 seconds. These beats commonly pass to the form of brief tetani, lasting some 1–4 seconds, which are a very characteristic result of strychnine in the crab. If poisoning is more severe or long continued, the heartbeats may become irregular or cease altogether, although retaining their amplitude to the last. The heart will usually recover if thoroughly washed.

The effects of strychnine upon nervous inhibition

Twelve experiments were performed, of which three were discarded because the electrodes had to be moved during the run, indicating poor setting or local injury to the nerve, while in three the heart failed before all the necessary tests could be car-

ried out. The remaining six indicate that strychnine, in concentrations of about 1:2500 (4×10^{-4}), is able to abolish the effect of the inhibitory nerves after perfusion for 30 to 60 minutes. The loss of response was verified in all cases by decreasing the coil distance of the stimulating inductorium by 5 mm. After a short period of washing, during which the beat usually showed a temporary depression, the inhibitory effect of the nerve was restored to or nearly to the initial value; in all cases the threshold after restoration was lower than the strongest stimulus used to verify the abolition of inhibition. In each of two experiments the abolition of inhibition in strychnine and its restoration after washing were repeated four times.

The effects of strychnine upon nervous excitation

The records of the effects of strychnine upon the effectiveness of the excitatory nerves to the heart are somewhat more reliable than are those for inhibition, since excitatory effects may frequently be obtained even after the heart has ceased to beat. Strychnine in concentrations of 1:2500 (4×10^{-4}) blocks the action of the excitatory heart-nerves, and this effect is fully reversible.

These experiments indicate that the cardiac regulatory nerves can be paralyzed by strychnine, as the somatic motor nerves presumably are, without causing the heart to cease beating. However, the anomalous character of the beat in the strychninized heart indicates that the intrinsic pacemaker neurones are affected considerably, though perhaps to a lesser degree than are the regulators.

Effects of Atropine Upon Nervous Inhibition and Excitation

Methods of study involving the use of inductorium stimulation, as in the work on the effects of strychnine described above, failed to reveal any effect of atropine on either the excitatory or the inhibitory nerves. Accordingly, confirmatory studies were made by the method of variable-frequency stimulation. Frequency-intensity threshold curves have been determined before and during perfusion with atropine, as well as after a period of washing.

The effects of atropine upon nervous inhibition

Atropine sulphate was perfused in concentrations of 10^{-5} and 10^{-4} for periods of 15–30 minutes before determining the frequency-intensity curves for the inhibitory nerves while the heart was still being perfused with the drug. The application of the drug followed, of course, the determination of such a curve while the heart was bathed with perfusion fluid. The cardio-inhibitory nerves of *Cancer* are not blocked by atropine, but are actually slightly augmented in their effects.

The effects of atropine upon nervous excitation

Atropine sulphate was perfused in concentrations of 10^{-6} to 10^{-4} for 15–30 minutes, as in the preceding experiments. The frequency-intensity threshold curves indicate that atropine in these concentrations has no significant effect upon the cardiac excitatory nerves. That a sufficient concentration of atropine (10^{-4}) was used is indicated by the fact that Welsh (1939b) found that atropine 10^{-5}

would largely block the effects of acetylcholine on the isolated heart of *Panulirus*, while atropine 10^{-4} is effective in this respect on the heart of *Astacus* (Davenport, Loomis, and Opler, 1940) and atropine 5×10^{-5} on the isolated heart of *Cancer magister* (Davenport, 1941).

Effects of Nicotine Upon Nervous Inhibition and Excitation

In view of the failure to block nervous inhibition and excitation with atropine, it was of interest to see how nicotine might affect these processes, since certain effects of acetylcholine ("nicotine-like" effects) are not abolished by atropine. A number of experiments were performed, using faradic stimulation. The results have been checked by variable-frequency stimulation. Nicotine, when first applied, has a strong augmenting effect on amplitude and rate of beat. The stimulatory effects wear off after a time, and, if the concentration of nicotine is high, depression of heartbeat ensues. Irregularity and grouping of beats are common in the early stages of nicotine depression; later stages are marked by infrequent beats or by an abnormal condition of intermittent tetanus. This last type of beat may be the result of a direct response of the heart muscle to stretching, as it could be seen in the records that the heart swelled noticeably between "beats." The crab heart adapts to nicotine, hence successive increases in the strength of drug applied produce relatively small effects.

The return of the heart to perfusion fluid after being in a strong nicotine solution frequently results in a temporary cessation of beat, following which the beat may be greatly accelerated as the nicotine concentration falls from a depressant to an excitatory level. During the period of complete stoppage, the excitatory nerve is able to function, an indication that this nerve is more resistant to the paralyzing effects of nicotine than is the cardiac ganglion.

The effects of nicotine upon nervous inhibition

In no case has inhibition been abolished by nicotine in concentrations of 1 : 10,000 to 1 : 1,000. Hearts responding to freshly applied nicotine by a marked frequency increase can be inhibited as readily as the normal heart, or as hearts which have been greatly depressed by strong concentrations of nicotine. The action of the inhibitory nerves can be studied only as long as the heart continues to beat, hence the effect of dosages of nicotine of strengths greater than 1 : 1,000 could not be determined.

The effects of nicotine upon nervous excitation

Nicotine in strengths of 1 : 1,000,000 to 1 : 500 has been tested, with inductorium stimulation of the excitatory nerves. The results fall into three classes, depending upon the strength of drug used and its effect on the rate of heartbeat. Nicotine in the lower concentrations (10^{-6}), or when first applied, has a marked positive chronotropic effect which may persist for some time. In certain experiments, stimulation of the excitatory nerve during this period of excitation has shown an augmentation of the effectiveness of the nerve as compared to its effectiveness upon the undrugged heart.

In intermediate concentrations of nicotine (10^{-5}), or in concentrations applied for a length of time sufficient to depress the heartbeat, nervous excitation is not

impaired. This fact indicates that the cardiac ganglion is more sensitive to nicotine depression than are the excitatory nerves. The complete stoppage of heartbeat that may follow the return of a heart to perfusion fluid after being in nicotine supports this view, since the excitatory nerves may stimulate a heart that has ceased its spontaneous beat under such conditions. In this situation the pacemaker ganglion has evidently lost its capacity for spontaneous discharge, although it must still be able to transmit impulses to the heart muscle via the neuromuscular junctions when properly stimulated. In considering the effects of nicotine, it must be kept in mind that the drug may not only block transmission at the nerve endings of the ganglion and extrinsic nerves, but may act on the ganglion cell bodies themselves, causing a loss of their rhythmical activity.

In high concentrations of nicotine the effects vary, depending upon whether the heart gives a slow beat of normal form or whether it passes into a state of irregular intermittent tetani. In the former case, stimulation of the excitatory nerve may still be able to rouse the heart to more rapid beating, but in the latter case the excitatory nerve appears to be ineffective.

Frequency-intensity threshold curves for the excitatory nerves showed no significant changes after perfusion of hearts with nicotine 10^{-6} or 10^{-5}. Higher concentrations of nicotine could not satisfactorily be tested in this way, since the hearts generally failed during the lengthy process of determining the curve. Thus even though there is evidence that certain concentrations of nicotine enhance the response to excitatory nerve stimulation, it has not been possible to show any increased effectiveness of lower rates of excitatory nerve stimulation.

The augmentation of excitatory nerve effectiveness by low concentrations of nicotine may perhaps be comparable to the action of nicotine on vertebrate autonomic ganglia, where low concentrations of the drug augment the effect of preganglionic stimulation. In high concentrations, nicotine blocks the passage of impulses at vertebrate autonomic ganglia. In the crab heart nervous excitation has been blocked by nicotine in high concentrations, but only in cases where the heartbeat is of abnormal form. Is this failure of excitation due to a blocking of the excitatory nerve, or is it due to overstimulation of the heart ganglion? The abnormal form of the heartbeat suggests that the function of the heart ganglion has been impaired. If we accept this explanation, we cannot say whether or not transmission at the endings of the excitatory nerves is being blocked.

To sum up the results of this study of the effects of nicotine on the cardio-excitatory nerves, low concentrations of nicotine augment the action of these nerves, and high concentrations appear to block their effects. But, because of the likelihood that the heart ganglion is at least as sensitive to nicotine as are the excitatory nerves, it is not possible to say whether the block occurs at the endings of the excitatory nerves themselves or in the heart ganglion. The evidence so far obtained points to the presence of a "nicotine-like" cholinergic mechanism involved in nervous excitation of the crab heart, but it does not yet allow us to decide between the cardio-excitatory nerves and the intrinsic heart ganglion as possible sites of this mechanism. Perhaps both possibilities are true, since any effects of nicotine observed could be explained on such a basis. The methods employed in this work do not enable us to distinguish between nicotine-block of the cardiac ganglion alone and simultaneous nicotine-block of the excitatory nerve and the heart ganglion.

Effects of Eserine Upon Nervous Excitation and Inhibition

In the crabs used in the present work, eserine (physostigmine sulphate Merck) in concentrations of 10^{-6} or 10^{-5} was found to potentiate markedly the effects of low concentrations of acetylcholine. Hence such concentrations of eserine were considered sufficient to inactivate a significant amount of the cholinesterase in the heart when perfused for 15 minutes or more. Such low concentrations of the drug do not, by themselves, visibly affect the heartbeat.

The effects of eserine on nervous inhibition

Eserine in concentrations of 10^{-6} to 4×10^{-5} did not augment the action of the cardio-inhibitory nerves when tested by inductorium stimulation, thus agreeing with results previously obtained on *Panulirus* (Smith, 1940–41), nor could any effect of eserine upon frequency-intensity threshold curves be detected.

The effects of eserine on nervous excitation

Since the effects of nicotine on cardio-excitation have made it appear at least possible that the excitatory nerves are cholinergic, the action of eserine on the effectiveness of these nerves is of importance. However, no success was obtained in producing an augmentation of excitation by the application of eserine. Inductorium stimulation did not produce detectable results, while frequency-intensity threshold curves show little more. Eserine either has no effect, or it actually makes cardio-excitation more difficult to obtain. It should be recalled that the concentration of eserine used was sufficient to augment the action of acetylcholine on the crab heart, but that it did not by itself cause any stimulation of the heart. Under such conditions, Wiersma and Novitski (1942) have found eserine to augment the the effects of nervous excitation of the crayfish heart, as determined by a study of heart rate.

Effects of Acetylcholine Plus Eserine Upon Nervous Excitation and Inhibition

It has been found that a low concentration of acetylcholine chloride (10^{-7}) has a long-continued stimulatory action upon the heart of *Cancer* if it is potentiated with eserine 10^{-5}. Neither drug has any marked or persistent effect if perfused alone. Such a mixture of drugs has been used to determine the effects of acetylcholine upon the action of the regulatory nerves. But as in the case of erserine alone, "protected" acetylcholine in low, stimulatory concentrations has no consistent or significant effect either upon the inhibitory nerves or upon the excitatory nerves. Because higher concentrations of acetylcholine gave effects too strong to be maintained over the period necessary for the type of observations made in this study, they were not used.

Discussion

The lack of effect of eserine and acetylcholine upon the excitatory nerves would seem to speak against the possibility that these nerves are cholinergic. Yet there are indications that the failure to detect eserine potentiation of the effectiveness of these nerves is not conclusive evidence of their non-cholinergic nature. Welsh (1940–41) has reported that while eserine augments the effects of low concentra-

tions of acetylcholine on the isolated lobster heart, it fails to potentiate, or even lessens the effects of acetylcholine stronger than 10^{-5}. It is probable that cholinergic nerves release at their terminations acetylcholine in small amounts but in high, sharply localized concentrations; while cholinesterase has been shown to be greatly concentrated at the motor end plates in vertebrate skeletal muscle (Marnay and Nachmansohn, 1938; Nachmansohn, 1939). In the case of vertebrate autonomic ganglia, Brown and Feldberg (1936) have shown that eserine can potentiate the effect of preganglionic stimulation so effectively that the ganglion cells are soon paralyzed by the accumulation of acetylcholine unless submaximal preganglionic volleys and low frequencies of stimulation are employed. If such a situation exists in the crab, it is possible that eserine may allow the accumulation of mediator substance in depressant concentrations. In view of the evidence of Wiersma and Novitski (1942) that eserine augments nervous excitation of the crayfish heart, a restudy of this matter in the crab would be desirable, employing a technique by which heart rate, uncomplicated by amplitude changes, could be observed.

The inability of the excitatory nerves to tetanize the crab heart, although this state may be produced by direct electrical stimulation, acetylcholine, nicotine, etc., raises the question of whether or not the excitatory nerves act in the same way or at the same point as these other agents. Prosser (1943) has shown that the systole of the neurogenic heart of *Limulus* is initiated by synchronous activity in the cardiac ganglion, while tetanus results from a continued asynchronous discharge. During the course of the present work it has been observed that crab hearts depressed by nicotine or strychnine often exhibit a series of very irregular abnormal beats which, during recovery, regroup themselves to normal systoles, apparently by a return of the several cardiac ganglion cells to synchronous activity. The short tetani that may replace the normal systoles under the influence of nicotine or strychnine may, likewise, possibly be caused by asynchronous discharges of ganglionic cells. Direct electrical stimulation, acetylcholine, and other agents capable of tetanizing the neurogenic arthropod heart may do so by throwing the cardiac ganglion into a state of asynchrony, but it would seem that the excitatory nerves are capable of producing only more closely spaced bursts of synchronous ganglionic activity.

Since other evidence so far accumulated supports the view that the heart ganglion is cholinergic, and since the lack of effect of atropine upon normal heartbeat indicates that the ganglion does not exert a "muscarine-like" action on the heart muscle, we may consider the possibility that the ganglion has a "nicotine-like" effect at the neuromuscular junction. It will be recalled that, in its action on different vertebrate tissues, acetylcholine has a variety of effects, which may be broadly classified into "muscarine-like" effects that can be blocked by atropine, and "nicotine-like" effects that can be blocked by high concentrations of nicotine. These classifications of acetylcholine action apply, strictly, to the reactions of vertebrate tissues, and it is possible that the reactions of invertebrate tissues do not fall into identical categories. Hence, the fact that the crustacean heart ganglion does not appear to exert a "muscarine-like" action upon muscle does not prove by elimination that its effects are fully comparable to the "nicotine-like" action of certain cholinergic nerves in vertebrates. For the present, however, we may consider that the cardiac ganglion transmits impulses to the heart muscle by a cholinergic mechanism having a somewhat "nicotine-like" effect.

If it is true that both the ganglion and the excitatory nerves are of the same pharmacological nature, this fact renders useless our attempts to demonstrate a nicotine block of the excitatory nerves. As pointed out above, the ganglion seems more sensitive to nicotine than are the excitatory nerves. Paralysis of the ganglion removes an essential link in the chain of impulses which pass from regulatory nerve to heart muscle in nervous excitation. With the ganglion out of action, the present methods do not reveal whether or not the excitatory nerves are blocked by high concentrations of nicotine, but the positive evidence so far obtained points to a cholinergic "nicotine-like" action of the cardio-excitatory nerves upon the heart ganglion.

Summary

1. The heart of *Cancer irroratus* has been perfused *in situ*, the beat recorded mechanically, and the inhibitory and excitatory nerves stimulated while the heart was perfused with various drugs.

2. Nervous inhibition of the heart induced by stimulation of the inhibitory nerves is usually total with no inhibitory after-effect.

3. Nervous excitation of the heart induced by stimulation of the excitatory nerves frequently shows a stimulatory after-effect.

4. The cardio-inhibitory nerves appear to be more effective than the excitatory nerves, but each type of nerve can modify to some extent the effectiveness of the other.

5. The excitatory nerves are effective when stimulated at frequencies ranging from 200–300 per second down to 10 per second or less.

6. The inhibitory nerves produce total inhibition when stimulated from 15–20 to 100–200 times per second. At frequencies extending above and below this range, partial inhibition may be obtained.

7. Strychnine, in concentrations of 1 : 2,500, blocks reversibly the action of both excitatory and inhibitory nerves.

8. Atropine is without effect upon excitatory and inhibitory nerves, as well as upon the heart ganglion in concentrations sufficient to abolish the action of applied acetylcholine.

9. Nicotine, in low concentrations, augments the effectiveness of the excitatory nerves as well as of the heart ganglion.

10. Nicotine in high concentrations may block the passage of impulses from cardiac ganglion to muscle; hence it could not be determined with certainty if nicotine blocks the excitatory nerves.

11. Neither eserine alone, nor with acetylcholine in stimulatory concentrations, augmented cardio-inhibition or excitation. It is suggested that the lack of effect of eserine upon the excitatory nerves deserves restudy.

12. It is probable that the excitatory nerves are cholinergic, and exert a somewhat "nicotine-like" effect upon the heart ganglion, which in turn exerts a "nicotine-like" action at the neuromuscular junction.

13. No light has been thrown on the pharmacological nature of the cardio-inhibitory nerves.

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